STERILITY AS THE RESULT OF HYBRIDIZATION AND THE CONDITION OF POLLEN IN RUBUS[1]

CARL SHERMAN HOAR

(WITH PLATES X–XII)

One of the first well authenticated records of the possibility of crossing species dates from 1694. In that year CAMERARIUS (1) in a letter to VALENTINE mentions the effect of crossing the hemp (*Cannabis*) with the hop (*Humulus*). However, it was not until 1760 that KÖLREUTER (2) made the first plant hybrid which was to be of use to science. He crossed *Nicotiana paniculata* ♂ and *Nicotiana rustica* ♀. In stating his conclusions he recognizes the relative fertility of hybrids from crossed varieties as compared with the relative sterility of hybrids made between distinct species.

As a result of KÖLREUTER'S research much interest was aroused among plant breeders as to the rôle of hybridization and as to the conditions which lead to sterility. Such men as KNIGHT (3) and HERBERT (4) will, perhaps, illustrate the diversity of opinion which sprang up. KNIGHT, on the one hand, held that hybrids from parents of distinct species were sterile, while, on the other hand, the varietal crosses were fertile (an opinion quite like that of KÖLREUTER). HERBERT, on the contrary, found that hybrids between different species are frequently fertile, a fact which he interpreted as signifying that the parents had branched from the same main stock. FOCKE (5) also has pointed out the fact that no peculiarity of hybrids has attracted so much attention as the lessening of the power of reproduction, and that crosses between more distantly related plants are more sterile even up to entire sterility. Other views have been expressed since, some favoring one side and others being equally sure that the contrary is true. However, though this diversity of opinion is prevalent even to the present time, nevertheless it seems clear that sterility is a common characteristic of hybrids.

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

Sterility may be brought about in several different ways, both physiologically and morphologically. My purpose is not to enter into the subject of physiological sterility, which is of much more interest economically than it is from the evolutionary standpoint; and, accordingly, I shall confine my attention entirely to the morphological side.

One of the most obvious types of morphological sterility is indicated by the sexual organs of the flower. Often as the result of hybridization the anthers and pistils are abnormal, and still more often, even when these are normal, we find a large percentage of sterile ovules present. The pollen shows this type of sterility especially well. I have records as far back as 1832 in which morphologically sterile pollen has been recognized as a criterion for hybridization. In that year DUTROCHET (6) wrote a letter to the Academy in which he attributed the sterility of hybrids in part to the imperfections of pollen. He refers to the flower of a hybrid cherry tree, resulting from the union of *Prunus cerasus* and *P. avium*, whose "stamina" formed a compact mass in which the pollen did not divide into pollinic or fertile dust as in the case of fruitful trees. This appears to be an extreme case, and yet it may rightfully be classed as a type of morphological sterility. GÄRTNER (7), in his well known essay, also refers to pollen sterility as a criterion for hybridization. Again, according to DUCHARTE (8), NAUDIN observed that the degree of fertility of hybrids was *en rapport* with the number of normal pollen grains formed in the hybrid.

Since the discovery of MENDEL'S work (9) a great impetus has been given to plant breeding, and we find many scientists entering into the problems opened up thereby. Most of the work done has been in regard to the inheritance of factors and to the working out of Mendelian laws of heredity. However, several observers have given their attention to the question of the pollen condition. ROSENBERG (10) discovered that in an apparently sterile hybrid sundew (*Drosera longifolia obovata*) the young pollen grains mostly lost their contents, while the embryo sacs usually did not develop beyond the binucleate stage. DETLEFSEN (11), working upon the animal side, has shown that a partial abortion of gametes occurs in the male progeny of a *Cavia* species cross.

Other interesting facts have been brought out from the cytological standpoint. Several investigators have worked at pollen sterility from this side, and though it is not my purpose to go into any prolonged discussion of the matter, yet it may be well to give a brief account of what has been done. JUEL (12), working with a hybrid known as *Syringa chinensis*, noticed that all the pollen was abortive and that abortion did not take place until after the formation of tetrads. TISCHLER (13), through his research upon the same hybrid, and also upon hybrids of *Ribes*, found that pollen abortion seemed to take place usually after the formation of tetrads. He also made a study of hybrids from a *Bryonia* species cross made by CORRENS, and found that the tetrads were formed but that the pollen generally aborted, as did also the embryo sacs. He ascribes the frequent sterility of mutants, accompanied by irregularities similar to those of hybrids during reduction, to the disturbance of the idioplasm. GEERTS (14), in working out the cytology of *Oenothera Lamarckiana* of DEVRIES, determined that one-half of the pollen from each tetrad aborted, and also that one-half of the embryo sacs did not develop. BELLING (15) speaks of this behavior as being unique, since, as he states, "these Oenotheras cannot be considered hybrids." This statement is worthy of note when taken into consideration with views recently expressed, not only about *Oenothera* as a genus, but concerning the Onagraceae as a whole. OSAWA (16) has recently shown that in the case of the Unshu (Satsuma) orange, although this is not known to be a hybrid, yet nearly all the pollen aborts and the abortion takes place only after the formation of tetrads.

As can readily be seen, all these writers emphasize the fact that the abortion of pollen seems generally to take place only after the formation of tetrads. DORSEY (17) not only points out that this seems to be the case with the genus *Vitis*, but he also distinguishes two kinds of abortion. To quote directly:

> In the formation of sterile and fertile pollen of the grape the heterotypic and homotypic divisions and the division of the microspore nucleus take place normally. Sterile pollen in the grape results from degeneration processes in the generative nucleus, or arrested development previous to mitosis in the microspore nucleus. When degeneration begins early after the division of the microspore nucleus, both the generative and vegetative nuclei may remain normal.

Before leaving the general question in regard to the formation of sterile pollen, I should like to call attention to another phase upon which no great amount of work has yet been done. This is in regard to Mendelian research. BRAINERD (30), in one of his articles concerning hybrids of *Viola*, describes violets which he has cultivated and which he knows to be hybrids from their external characters. BATESON (22) makes this statement: "Such an experiment raises the hope that sterility consequent upon crossing, the most obscure of all genetic phenomena, may become one of the possibilities of Mendelian research." JESENKO (19), using a cross between wheat and rye, was able to obtain one hybrid plant with pollen mostly shrunken and sterile. After several artificial crosses he was able to obtain a second generation hybrid which was apparently quite fertile. However, when he repeated the experiment on a larger scale, he obtained several first generation hybrids, but could obtain none of the second generation except through back-crosses. He now thinks that the first second generation plant which he obtained was of such a nature. SUTTON (20) also has done work in crossing varieties and species of cultivated peas, together with a wild pea of Palestine (*Pisum humile*), and his results have been confirmed by BATESON (22). Although he found the first generation hybrid to be either entirely or partially sterile, yet when he obtained second generation plants, he found them to be fertile. This may, perhaps, be explained by the fact that he used plants close in relationship and hence more compatible.

Within a few months a study of pollen sterility has been made by BELLING (15) in which he has attempted to show that certain ratios prevail. His work has been carried on in Florida, chiefly with 3 species of *Stizolobium*. The first, the Florida velvet bean (*Stizolobium deeringianum* Bort.), he states is of unknown origin, but is very commonly known as "a large ornamental tropical climber." The second, the Lyon bean (*Stizolobium niveum* Roxburgh), is commonly grown in the Philippines and elsewhere in South Asia. The Yokohama bean (*Stizolobium hassjoo* Piper and Tracy), which he takes for the third, is common upon the island of Japan. He has worked more especially with these, but some work, though only with the first generation hybrid, has also been done by him with the China bean (*Stizolobium niveum* var. ?), commonly considered as a variety of the Lyon bean. Perhaps it is not well to go into more detail, but rather to allow the reader the privilege of consulting the article for fuller details. His conclusion is that, generally speaking, 50 per cent of the pollen of the F_1 generation loses its contents and becomes sterile. A similar degree of sterility seems also to hold for the embryo sacs. Where the experiment was carried to the F_2 and F_3 generations, it was found that the offspring of the fertile half all bred true to fertility, while those of the sterile half all persisted in having 50 per cent sterile and 50 per cent fertile. The ratio here, as the investigator points out, is 1:1. As to the value of the results obtained, I am not in a position to judge. However, the fact that the investigator is dealing with the haploid generation instead of the usual diploid generation lends a new interest to the matter and opens up a possible new field of research.

Thus far I have attempted to give a general idea of some of the work done heretofore upon sterility in pollen, and its relation to hybridization. The work of DEVRIES and his "Mutationstheorie" resulting therefrom have given a great impetus to a search for the true means of evolution in living matter. The Darwinian idea that species arise through a gradual loss or gain of characters and the survival of the fittest of these has been very seriously attacked. The "sporting" condition which DARWIN recognized as occurring sometimes, but which he considered to be of far less importance than the changes brought about gradually, has been made to play a very much more important part by DEVRIES' mutation idea.

DEVRIES bases his theory primarily upon the much discussed species *Oenothera Lamarckiana*, which he had been growing for several generations in his garden, and of whose origin he is in no way certain. As I have already shown, sterile pollen, though not necessarily present in hybrids, is a common character. Recently JEFFREY (21) has shown that, so far as his examination has extended, morphologically sterile pollen does not occur, except in a percentage small enough to be accounted for by unfavorable conditions, in plants which are either monotypic or are isolated either in a geographical sense or through the time in which the flowers are matured. He has shown, moreover, that below the angiosperms a comparatively small amount of sterility occurs, and that in the gymnosperms it is almost entirely absent. On the other hand, the angiosperms present a very different aspect. Certain families of the last have long been a puzzle to the systematist. BATESON (22) points out that in *Oenothera Lamarckiana* a large percentage of sterile pollen is present. Other geneticists and cytologists, including DEVRIES himself, have noted this, but perhaps BATESON was the first to attribute it to hybridization. JEFFREY (21) has shown that the condition of abortive pollen is characteristic not only of *Oenothera Lamarckiana*, but of the *Oenothera* species in general, and that, moreover, the entire family of the Onagraceae is so characterized. Because of this he considers that DEVRIES' "Mutationstheorie," in so far as it is based upon *Oenothera Lamarckiana*, is lacking in justification. In other words, in order to establish such a theory securely, one must deal with plants and with animals which are species in the fullest sense of the word. Geneticists have long recognized the variability of hybrids, and hence plants with any trace of hybridization should not be used as a basis for a theory of such fundamental importance as that promulgated by DEVRIES. In a recent article DEVRIES (23) has attempted to refute JEFFREY's contention that pollen sterility is a criterion for hybridization. He states that, from such a viewpoint, we might consider the abortion of the 3 mother cells in the formation of the mature egg, which customarily takes place in the angiosperms, as a criterion for their hybrid origin. Again, he cites the case of certain sedges in which ordinarily 3 of the pollen mother cells abort and we find their remnants clinging to the mature pollen grain. His examples appear to me to be chosen from conditions which have no direct relation to the situation found by JEFFREY.

After what I have just said it is fitting, perhaps, to add a few words as to what really helps to make up a true species. In attempting such a discussion I realize that I am treading upon more or less forbidden ground; however, I shall give views which seem to me to be well supported by the observations of experts. There are those who would separate the idea of species entirely from that of a hybrid, but, on the other hand, we find a large number of prominent biologists who hold a different view. In an article by MEEHAN (24) I find the following reference to LINNAEUS: "The great father of botany does not seem to have much, if indeed any, idea of sterility in his comprehension of the term. He believed that a large number of plants which he regarded as true species and to which he gave specific rank and specific names originated as hybrids. Thus we have *Trifolium hybridum*, *Trigonella hybrida*, *Campanula hybrida*, etc., among a large number of similar Linnean names." OLIVER (25) remarks that "natural hybrids have been known for a long time and we have every reason to believe that they have existed when and where conditions were favorable for their production." Recently GATES (26), in an article upon *Oenothera Lamarckiana*, writes as follows: "Crossing must have taken place in the ancestry of *Oenothera Lamarckiana* as well as in other flowers which are open pollinated. Among open pollinated plants (and the same is probably true for animals) there is no such thing as a 'pure' species, but, rather, many interbreeding races, whose combinations vary from generation to generation, make up the population." Upon the animal side views similar to this have been expressed. In an article recently published GEROULD (27) has shown how important a rôle hybridization has shown in forming species, and its relation to mutation.

I might cite other references (such as BATESON **22**, JANCZEWSKI **28**, CASTLE, BACOT, PROUT, etc.) to show how various biologists consider the term species. However, perhaps the foregoing will be sufficient to give some idea of the difference in opinion concerning the matter. Suppose we grant that two such ideas of a true species can exist together, then are the angiosperms, or at least many of them, species in the same sense as those which we find among the gymnosperms? In other words, is not the greater abundance of angiosperms as compared with the number of gymnosperms to be accounted for, to a large extent, through the formation of natural hybrids which breed true and have characters distinct enough to make them good species when viewed from the systematic standpoint? Certain families like the Onagraceae, Violaceae, Solanaceae, Rosaceae, etc., have long presented great difficulties to botanists.

Recently a specialist in *Oenothera* (BARTLETT 29) has published an article in which he makes what he calls "12 elementary species of *Onagra*," 5 of which are made out of our common evening primrose (*Oneothera biennis*). This is surely a perplexing condition. BRAINERD (30), who has made a very careful study of the species of *Viola*, both in the field and under controlled conditions, has met with a very disconcerting situation. He finds a difference in the classes of hybrids. When hybrids are made between nearly related but distinct species, they have characters intermediate between the unlike characters of the parent forms. They are stable, breeding true from generation to generation, and showing a very vigorous growth, but are always more or less sterile. On the other hand, when crosses are made between species which are more or less doubtfully distinct, or between races, or between a species and a variety, there is usually quite a different result. In the first place, the offspring of such crosses have not intermediate characters, but recombinations of the unlike characters of the parent forms. As a result, the individual plants are dissimilar, some reverting to one or the other parent form, and others presenting the "sporting" characteristics. Another very important characteristic difference is that we do not usually find the fertility damaged either in the first or in subsequent generations. He speaks of these as "the so-called Mendelian hybrids, the despair of the systematist but the vantage ground of the breeder of new and useful 'varieties.'" Finally, he cites the instance of several species of *Aquilegia* which he allowed to grow indiscriminately together, and which in a short time gave off 5 hybrids. This case is similar to that of the violets which, owing to the greater amount of the cleared land, can be greatly increased in range and hence have a much greater opportunity for hybridization.

In connection with BRAINERD'S remark concerning his so-called Mendelian hybrids it seems well to quote a few lines from another. CORRENS (31) was one of the first to rediscover MENDEL. He evidently from the first appreciated certain limits to the scope of MENDEL'S laws.

Inzwischen hat mir ein Aufenthalt in den Alpen Gelegenheit gegeben, eine Anzahl von Bastarden zwischen unzweifelhaften Arten zu untersuchen

(*Cirsium palustre*×*spinosissimum*, *Cirsium heterophyllum*×*spinosissimum*, *Achillea moschata*×*nana*, *Achillea macrophylla*×*moschata*, *Carex echinata*×*foetida*, ferner *Melandryum album*×*rubum*, hier eine vollkomme gleitende Reihe von einer Art zu andern). Dabei ist mir zweifelhaft gebleiben, auch nur bei einzigen dieser Bastarde in einem einzigen Merkmalspaar ein wirkliches Dominiren verkommt, ganz sicher ist, dass in fast allen Punkten, in denen die Eltern differiren, der Bastard die Merkmale beider Eltern zeigt, jedes abgeschwächt, wenn auch in verscheidenem Grade.

Other paragraphs might be cited which bear more or less upon the subject, but I shall cite but the closing line of the article.

Die Aufdeckung der Mendel'schen Regeln wird also kaum dazu beitragen, dass von jetzt ab Speciesbastarde und Rassenbastarde in einem Topf geworfen werden, und man statt dessen nur von Mono- Di- etc., Polyhybriden sprechen wird, sie wird im Gegentheil wohl der Anfang für eine schärfere Trennung der beiden sein.

The Rosaceae, which is the last of the families mentioned as being a perplexing problem for the systematist, has among its many genera several whose species are very much unsettled. Of these *Crataegus*, *Rosa*, and *Rubus* may be mentioned as distinctive. With these 3 genera there have been great changes wrought within the last few years. Taking *Crataegus*, for example, we find a very interesting account of its present condition by BROWN (32). When GRAY'S *Field, forest, and garden botany* was published in 1857 there were listed in it but 12 species and 2 varieties. Again, in GRAY'S *Manual of botany* of 1867 we find only 10 species and 4 varieties given. It was not until after the publication of CHAPMAN'S *Flora of the southern United States* in 1897, which gave but 15 species within its range, that we find any great increase in the number of species. Since then many species have been described. In fact, since 1896 there have been, according to BROWN, 864 species and 18 varieties described. Where will this species making end? As BROWN states, "Why did not the systematists discover this great number of species before? Have they been recently hybridizing? Undoubtedly a part of the great increase may be explained by a more intensive study upon the part of the systematic botanist, but does this account for it all?" BRAINERD, in reply to BROWN'S inquiry, states that "the Rosaceae of all orders is the *most predisposed to hybridize. Rosa, Rubus, Geum, Amelanchier,* and *Malus* are notorious for the forms resulting from interbreeding. By analogy we should expect the same condition of things in *Crataegus*."

Rubus presents a condition of things quite like that of *Crataegus*. Although GRAY'S *Manual* of the last edition contains 38 species all told, yet the authors carefully state that in *Eubatus* we have "a group of great taxonomic difficulty, in which many species have been recently proposed. Of these the better marked have been here freely included, but without entire confidence that the future intensive study may not show them to be intergradient and perhaps in many cases hybrid forms." Because of this great variation and because of the uncertainty as to which are and which are not true species, I have selected this genus in which to make a careful study of the pollen conditions. However, before proceeding to the results of my research, it seems best to give a brief historical account of *Rubus* and the work done upon it up to the present time.

Rubus, like *Crataegus*, has had an almost miraculous growth in number of species within a few years. Only 18 years ago GRAY'S *Manual* recognized but 3 species and 2 varieties for New England, and, as BRAINERD (33) remarks, 2 of these specific names were wrongly applied. In 1898 BAILEY (34) published his *Evolution of native fruits* in which he recognized 13 species of *Rubus* of the *Eubatus* group for New England. Since that time BLANCHARD has published detailed descriptions of 40 or more forms, most of which he has considered as species. Many of these, as I shall show later, appear to be of hybrid origin from a study of the pollen; but from an external study alone he came to the conclusion that they were worthy of specific rank.

In 1911 BICKNELL (35), in an article entitled "Have we enough New England blackberries?" makes the following statement: "*The least unstable species* (*here*) *possess some kind of ready pliancy which answers often with marked emphasis to slightly changed conditions of growth, and further, all the species by some freely practised method of versatility acquire variously in combination with their own proper characters the features of associated members of the group*."[2] He adds that these facts impart an extraordinary natural variability

[2] The writer is responsible for the italicizing.

and undoubtedly also *a facility in hybridizing which is, perhaps, not exceeded in any of our flora.* In speaking of BLANCHARD'S species he remarks that "probably 60 per cent of these are synonyms, while the remainder, with possibly a few exceptions, *appear to disclose themselves as scarcely doubtful hybrids.*" In his mind there are about 11 species of *Eubatus* which alone can be considered valid, while the others are hybrids. He also gives a list of their names as he believes they should appear. BRITTON, in the new edition of the *Illustrated flora*, recognizes but 10 species, none of BLANCHARD'S naming being mentioned even as synonyms. More recently RYDBERG in his monograph of *Rubus* has allowed 27 of the *Eubatus* group for the northeastern United States, including 7 of BLANCHARD'S naming and 4 of BAILEY'S. Of the remaining 30 and more of BLANCHARD'S names, 9 are disposed of as synonyms and 24 as hybrids.

BRAINERD (33) in his recent article upon *Rubus*, which he has published after years of study and from which I have drawn many of my historical data, remarks: "No mere herbarium botanist is qualified to disentangle the perplexing intricacies of a group of plants like *Rubus*. The days of random guessing at parents are past or should be." He then states what to him are the proper methods of procedure in determining a true species, and gives a list of 9 "probable good species" in Vermont.

The phenomenon of variability in *Rubus* appears not to be confined to American species, but to be characteristic of the genus everywhere. HOOKER in his *Flora of the British Isles* names 41 forms as species, while GARKE'S *Flora von Deutschland* contains more than 60 species. FOCKE (5) makes the following interesting statement:

> Die Gattung *Rubus* ist ungemein formenreich und zerfällt in eine Anzahl verscheidener wohl charakterisirter Untergattungen. Einige Gruppen bestehen aus aüsserst zahlreichen nahe verwandten Racen, welche die Grenzen zwischen den durch wesentliche Charactere gescheidene Arten oft völlig vermischen. Auch viele sonst gut umgrenzte Arten sehr formenreich natürliche Hybride scheinen innerhalb vieler Artengruppen leicht zu entstehen, wahrscheinlich gehen haufig aus ihnen neue constante Racen hervor.

During the summer of 1915 I was able to obtain from the Harvard Botanical Garden and from the Arnold Arboretum buds of about 40 species of *Rubus*. In every case I picked those buds which were about to open, and in this way I was able to obtain pollen grains which were mature. If these were morphologically good, they were large, round, and very full of protoplasmic substances. Material, on the other hand, in which the pollen had not matured normally showed it to be shrunken and empty, having lost all or most of its protoplasm and having but a vestige of the nucleus present.

During the following winter I made a study of the pollen condition present in these buds, the method in most instances being as follows. Owing to the fact that perfectly formed pollen is very densely filled with protoplasmic substances, no harm resulted from killing directly with 95 per cent alcohol. Other more tedious methods were tried, but no better results were obtained. The buds were then pricked, the air pumped out, and the buds thoroughly dehydrated in absolute alcohol. After this they were placed in a 2 per cent solution of celloidin dissolved in equal parts of synthol and ether. The grades of celloidin were raised 2 per cent every 12 hours, until 16 per cent was reached. Then small pieces of dry celloidin were added at 12- or 24-hour intervals, until a sufficient density was reached. When such a condition was obtained, the buds were removed one at a time and allowed to harden in chloroform for about 12 hours. They were then placed in equal parts of 95 per cent alcohol and glycerine. When completely hardened these were mounted upon blocks in a 6 per cent solution of celloidin and cut upon the microtome. After being cut and stained in Haidenhain's iron alum hematoxylin and counterstained in safranin, they were mounted in balsam. Such a method allows the observer actually to see the pollen as it occurs in the anther, and by so doing it gives one a more accurate idea of the true condition.

The genus *Rubus* in the last edition of GRAY's *Manual* is divided into 5 groups with FOCKE as an authority for their names. Of the first group, which is called the IDAEOBATIS group, I have 3 different forms (*Rubus strigosus* Michx., *R. neglectus* Peck, and *R. occidentalis* L.). It is here made a very small group, having but 3 well authenticated species. *R. strigosus* Michx. (fig. 1) is given as a synonym for a variety of *R. idaeus* L. I find the pollen condition to be nearly perfect and hence to give every indication that, from a morphological basis at least, the plant should be given a specific rank. *R. neglectus* Peck (fig. 2), on the other hand, shows a large percentage, perhaps 50 per cent, of sterile pollen. This condition goes very well with the remark made in the *Manual* that it probably is a self-perpetuating hybrid. Fig. 3 is an enlarged view of a portion of the field shown in fig. 2. *R. occidentalis* L., which is the well known black raspberry or thimbleberry, is given a specific rank in the *Manual* and the pollen condition indicates that such a position is tenable.

Of the second, or ANAPLOBATUS group, I have 2 members. The first, *R. odoratus* L. (fig. 4), the beautiful flowering raspberry, shows pollen almost universally good. Such a position would be expected from the late period in which it bears its flowers. Even should it be susceptible to crossing with other members of the genus, its lateness in regard to the opening of its buds would make it very hard to do so. *R. parvifolius* Nutt., on the other hand, although it is put here as a good species, contains a large percentage of pollen which is clearly sterile.

I have been unable to obtain any material of the members of the third (CHAMEMORUS) and of the fourth (CYLACTIS) groups, including in the *Manual* but 3 species.

It is the fifth or EUBATUS group which is by far the largest and has given the most trouble. In studying these I have found several whose pollen is nearly perfect, so nearly so in fact that the few shrunken grains which do appear may be accounted for by some unfavorable physiological condition. By far the greater number, however, show a greater or less degree of sterility. For convenience I have divided these last into those members ranging from sterility of about 25 to about 75 per cent; and also those which show a range greater than 75 per cent.

Among those with good pollen appear the following: *R. alleghaniensis* Porter, *R. setosus* var., *R. fruticosus* L., and *R. amnicolus* Blanch. *R. alleghaniensis* Porter (fig. 5) and *R. setosus* Bigel. (fig. 6) are given specific rank in the *Manual*. *R. fruticosus* L. (fig. 7), which is a European form, is suggested here as perhaps the same as *R. laciniatus* Willd., with the leaves less cut. *R. amnicolus* Blanch. (fig. 8) is placed together with *R. orarius* Blanch. as a synonym for *R. pergratus* Blanch.

Of those with a percentage of over 25 and less than 75 sterile I have *R. frondosus* Bigel., *R. recurvans* Blanch., *R. glandicaulis* Blanch., *R. Jecklyanus* Blanch., *R. orarius* Blanch., *R. arundelanus* Blanch., and *R. amabilis* Blanch. Of these GRAY'S *Manual* cites 4 as probably of specific rank, namely, *R. frondosus* Bigel. (figs. 9 and 10), *R. recurvans* Blanch., *R. glandicaulis* Blanch., and *R. Jecklyanus* Blanch. *R. orarius* Blanch. is placed together with *R. amnicolus* Blanch. as a synonym for *R. pergratus* Blanch. *R. arundelanus* Blanch. is considered as a synonym for *R. recurvans* Blanch. Finally, *R. amabilis* Blanch. appears under the same name as *R. canadensis* L. Comment upon this group is unnecessary, unless to remark upon the apparent perplexity its members have caused systematists. Clearly from the standpoint of sterility much hybridization is now occurring and has already taken place.

Of the last group, those with over 75 per cent of the pollen sterile, I have 4 specimens. These are *R. biformispinus* Blanch. (fig. 11), *R. Andrewsianus* Blanch. (fig. 12), *R. tardatus* Blanch. (fig. 13), and *R. peculiaris* Blanch. These all seem to have distinctive form, and from the standpoint of the systematist may warrant a species rank, at least they are so considered in the *Manual*. However, the pollen condition plainly points not only to hybridization, but to hybridization between species which must have been quite distinct and certainly quite incompatible.

Besides these forms I have 10 or more which are not included with the range of GRAY'S *Manual*. But one of these, *R. deliciosus*, (fig. 14), appears to be a good species when taken from the standpoint of absence of sterile pollen. I have *R. caesus turkestanicus*, *R. corylifolius*, *R. spectabilis*, *R. plicatifolius*, *R. multiformis* (fig. 15), *R. semierectus*, and *R. spectabilis plena* (fig. 16). One of the forms, *R. nobilis* (figs. 17 and 18), which is known as a hybrid, is almost entirely sterile, and, as one would expect, contains but a very few grains which can possibly be considered as viable.

Since my purpose has not been to attempt to place systematically the members of the genus *Rubus*, I have not given any account of the various characters upon which the systematists separate its species. Indeed it appears to be too hard for the systematist himself. Scarcely any two systematists agree as to which are and which are not true species. This is especially well shown by the large number of synonyms which one meets in any systematic work upon the genus.

However, I have found that sterility of pollen is a common characteristic throughout the entire genus. But few of the species indicate a perfect pollen condition and some are almost entirely sterile. In other words, the genus *Rubus* indicates to me a condition which appears to prevail in many angiosperms. JEFFREY (21) has already pointed out how prevalent sterile pollen is throughout the whole family of the Onagraceae, and other groups of plants are now being worked upon in this laboratory which seem to show a similar condition.

In closing, it seems to me that many of the species of the angiosperms are in a very different condition from that of others of the same group and from practically all those of the gymnosperms. They appear often to be natural hybrids which are formed, as BRAINERD has pointed out, as a result of a cross between distinctly separate species. Such crosses do not result in the segregation which Mendelian crosses usually exhibit, but rather in the blending of the characters of the parents. CORRENS (31) in his observations has noted such a situation in regard to certain plants. Such offspring may breed true to their respective characters and thus perpetuate a distinct form which, from a systematic point of view, is entirely of specific rank, but which should be treated by the plant breeder in an entirely different manner. Such forms usually have a large percentage of sterile pollen. Thus *Rubus* hybrids formed by distinct species are very sterile, in fact almost entirely so, while crosses of varieties which are closely related, and which hence are usually more compatible, may give almost no indication of their hybrid origin.

Thus it appears to me that the genus *Rubus* is one of those angiospermic genera in which the term "species" must be used in a different sense from that applied to many other members of the angiosperms, and practically to all of the gymnosperms. It is a group which is a puzzle to the systematist, and whose species will, perhaps, never be definitely established.

Conclusions

1. Sterility of pollen has long been recognized as a criterion of hybridization.

2. Crosses between distinct species have long been known to be more or less sterile and to behave differently from crosses between more closely related forms or varieties.

3. True species when crossed do not, in most cases, follow the laws of MENDEL, but tend rather to blend to form more or less constant types, often systematically recognized as species.

4. Many species of the angiosperms are species in a very different sense from those of the lower plants and of the gymnosperms in particular.

5. In some cases they are natural hybrids which have external characteristics distinct and constant enough to have specific rank from a systematic standpoint.

6. Although these species may be distinct from the systematic standpoint, yet they must be treated in a different manner from the standpoint of the evolutionist and the plant breeder.

7. Finally, the species of the genus *Rubus*, as shown from the pollen condition and also from external characters, clearly hybridize very frequently in nature, giving rise to constant forms often recognized as true species.

In closing I wish to express my sincere thanks to those in charge of the Arnold Arboretum and of the Harvard Botanical Garden for the privilege of collecting. I desire, also, to express my thanks to Miss DAY, librarian of the Gray Herbarium, for assistance in securing literature, and to others who have kindly aided me in carrying out my purpose. This work has been carried on in the Laboratories of Plant Morphology of Harvard University under the direction of Professor E. C. JEFFREY, and to him I am indebted for advice and assistance.

HARVARD UNIVERSITY

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EXPLANATION OF PLATES X–XII

FIG. 1.—*Rubus strigosus* Michx.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 2.—*R. neglectus* Peck: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 3.—*R. neglectus* Peck: more highly magnified view of fig. 2; ×300.

FIG. 4.—*R. odoratus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 5.—*R. alleghaniensis* Porter: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 6.—*R. setosus* Bigel.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 7.—*R. fruticosus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 8.—*R. amnicolus* Blanch.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 9.—*R. frondosus* Bigel.: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 10.—*R. frondosus* Bigel.: more highly magnified view of fig. 9; ×300.

FIG. 11.—*R. biformispinus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 12.—*R. Andrewsianus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 13.—*R. tardatus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×600.

FIG. 14.—*R. deliciosus:* transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 15.—*R. multiformis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 16.—*R. spectabilis plena:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 17.—*R. nobilis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 18.—*R. nobilis:* more highly magnified view of fig. 17; ×300.